Adenosine and Migraine

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ABSTRACT: ***Background:*** Adenosine is a powerful natural vasodilator that participates in the control of cerebral and meningeal blood flow. In this context, it could be involved in the pathophysiology of migraine, since it was previously reported that intravenous adenosine can precipitate crises in migraine patients. ***Methods:*** We have investigated circulating adenosine levels in 12 patients suffering from migraine without aura, during crises and in crisis-free periods, and have compared the levels noted to those of a population of 10 controls. To determine if there are interactions between adenosine and serotonin, we examined the effect of adenosine and antagonists on the uptake and the release of (^{14}C) serotonin by platelets. ***Results and conclusion:*** We have reached a dual conclusion: 1) during migraine headaches there is an increase (mean 68%) in circulating adenosine levels and this increase may participate in cephalalgia; 2) activation of A2 receptors by adenosine causes a dose-dependent inhibition of calcium-dependent serotonin uptake by platelets. This inhibition of uptake could participate in the rapid elimination of serotonin in migraine sufferers. As a result of this, the use of adenosine antagonists could be an effective complementary treatment for migraine.

RÉSUMÉ: Adénosine et migraine. ***Introduction:*** L'adénosine est un vasodilatateur naturel puissant qui intervient dans la régulation du débit sanguin cérébral et méningé. À ce titre, elle pourrait être impliquée dans la physiopathologie de la migraine. En effet, il a déjà été rapporté que l'adénosine administrée par voie intraveineuse peut précipiter une crise chez les patients migraineux. ***Méthodes:*** Nous avons étudié le taux d'adénosine circulant chez 12 patients souffrant de migraine commune, pendant et en dehors des crises, et nous avons comparé ces taux à ceux d'un échantillon de 10 témoins. D'autre part, nous avons étudié les effets de l'adénosine et de certains de ses antagonistes sur la captation et la libération de sérotonine marquée au carbone-14 par les plaquettes de migraineux afin de déterminer s'il existe des interactions entre l'adénosine et la sérotonine. ***Résultats et conclusions:*** La conclusion de notre étude est double: 1) il existe une augmentation du taux circulant d'adénosine au cours de la céphalée migraineuse (moyenne 68%) et cette augmentation peut contribuer à la céphalée; 2) l'activation des reecepteurs A2 par l'adénosine provoque une inhibition, qui est fonction de la dose administrée, de la captation de la sérotonine par les plaquettes. Cette inhibition de la captation pourrait contribuer à l'élimination rapide de la sérotonine chez les migraineux. De ce fait, l'utilisation d'antagonistes de l'adénosine pourrait être un traitement adjuvant efficace dans la migraine.

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Adenosine is a powerful natural vasodilatator active in the distal vascular territory, and also in carotid territories.[1,2] This vasodilation is secondary to the binding of adenosine to P1 purinergic receptors, which include sub-types A1 and A2. The stimulation of adenosine receptors causes a relaxation of the smooth vascular muscles, leading to vasodilation.[3] Purinergic receptors are present in large numbers in the nerve endings of cerebral and meningeal vessels[4] and are also present on platelet membranes.[5] Adenosine could be involved in the pathophysiology of migraine for several reasons: because it is a powerful natural vasodilatator which regulates cerebral blood flow[1,6] because it has an algogenic action since it favors the release of prostaglandins[7] and because its infusion has been shown to induce intense headaches.[6] It was also reported that intravenous adenosine administration can precipate migraine crises in migraine patients.[8] Although the application of transcutaneous vibratory stimuli can relieve certain types of facial pain such as neuralgia, vibrations aggravate migraine headache.[9] It is now well established that vibratory stimulations induce the release of adenosine.[10] In light of these data, the study had a dual aim: to measure the level of circulating adenosine in patients suffering from migraine without aura during crises and in migraine-free periods and to compare these data to those noted in controls. In addition, in order to define interactions between adenosine and serotonin, we examined the effect of adenosine and antagonists on the calcium-dependence uptake and the release of (^{14}C) serotonin by platelets.

Patients and Methods

Twelve patients (9 women and 3 men), as well as 10 headache-free controls (6 women and 4 men) were included in the study (see Table 1). All patients suffered from migraine without aura, in conformity with the Headache Classification

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Table 1: Comparison of Adenosine Plasma Levels in 12 Patients During and Between Migraine Attacks and in Ten Control Subjects. Means and Standard Deviations (SD) are Given.

PATIENTS			CONTROLS	
Age/Sex	Between migraine attacks	During migraine attacks		Age/Sex
19/F	150	310	210	21/M
22/F	60	110	90	25/M
25/F	260	540	220	27/F
28/F	247	290	350	31/M
29/M	176	290	217	33/F
29/F	504	760	160	35/F
33/F	108	227	123	41/M
38/M	120	300	215	49/F
41/M	240	320	180	31/F
49/F	90	160	340	28/F
19/F	210	320		
45/M	360	630		
Mean = 31.4	210	354	210	32
SD = 9.9	125	191	83	8.1

Committee[11] and had at least one acute attack per month (mean 2.3, SD = 1.3) for at least five years. Being under the necessity to stop working was the headache severity criterion. Excluded were smokers, those treated with papaverine, dipyridamole, indomethacin or salicylate, as well as those having ingested coffee or tea within 48 hours prior to sampling. Those who could not stop coffee because of dependence were also excluded. Finally, all treatments were stopped two weeks prior to sampling. Patients were invited to note the precise beginning of the cephalalgia and to go as quickly as possible to the hospital. All patients gave their written consent after being informed of the aims and modalities of the study according to the Helsinki Convention. Our project was approved by the local ethical committee (CHU Timone).

Experimental Procedure

Venous blood was drawn three times on different days. The first sample (10 ml) was taken 1 hour after the beginning of the headache and before any treatment. The second (10 ml) was taken at least 72 hours after the end of the attack, both of them to assay blood adenosine levels. The third (30 ml) was taken during a crisis free period to prepare platelet-rich plasma (PRP). Patients were recruited at consultation for pain (Marseilles North University Teaching Hospital). Blood samples were taken during a hospital stay and were assayed in the Biochemistry Laboratory.

Adenosine Assay

Adenosine (crystallized, 99% pure), adenosine deaminase (calf intestine, specific activity 200 IU/mg) and dipyramidole (5 mg/ml) were obtained from Boehringer Mannheim (France), inosine (99% pure) and (Arg)8 vasopressin, α, β-methyleneadenosine-5'-diphosphate (AOPCP) and deoxycoformycine were obtained from Sigma. 9-Erythro (2-hydroxy-3-nonyl) adenine (EHNA) was obtained from Burroughs Welcome. Methanol and other reagents were obtained from Merck (France). The reversed phase chromatography column (Merck LIChrospher C18, 250 x 4 mm) was obtained from Merck, France.

Samples were analyzed chromatographically by high performance liquid chromatography (Kratos HPLC 4000) fitted with a 1 ml loop. Absorbance was measured at 254 nm and eluted peak areas were measured with a Shimatzu Chromatopac C-RCA integrator.

Sample collection and treatment have been previously described.[12] Briefly, total venous blood (10 ml) was drawn simultaneously with a stopping solution, in Vacutainer tubes under vacuum. This method enabled the blood sample to be rapidly mixed with 20 ml of stopping solution, which prevents adenosine uptake by red cells.[15] This solution was composed of 0.2 mM dipyridamole, 4.2 mM Na2 EDTA, 5 μM EHNA, 79 μM AOPCP, 0.9% NaCl. The sample plus stopping solution was centrifuged at 2500 g for 10 min and the supernatant was deproteinized by adding 2 ml of 70% perchloric acid before centrifugation (2,500 g for 10 minutes). The supernatant was lyophilized and redissolved in 1 ml of 50 mM sodium phosphate buffer (pH 4). The resulting solutions were filtered by centrifuging in a Millipore Ultrafree-MC 0.45 μm filter before being chromatographed.

HPLC

The technique has been previously described.[12] Briefly, plasma adenosine was assayed by reversed phase HPLC on Merck C18 columns (250 x 4 mm). The column was equilibrated with 50 mM sodium phosphate buffer (pH 4). The sample was injected and was eluted with a methanol gradient (0 to 46% methanol in 40 minutes) at a flow-rate of 1 ml/min. Adenosine was identified by elution time and by incubation with adenosine deaminase which increases the inosine peak and decreases the adenosine peak (see 14). Adenosine was quantified by comparing the peak areas given by known quantities of adenosine.

PRP Preparation

Blood samples (30 ml) were collected on sodium citrate and centrifuged at 600 g for 10 minutes. Four ml of platelet-rich plasma (PRP) were then obtained from each patient or control, and added to 800 μl of 0.5% trisodium citrate, pH 6.5.

[^{14}C] Serotonin Incorporation Into PRP

PRP was separated into eight 500 μl samples (2 x 4 samples for duplicate assays). The following were added to two samples in the following order: 100 nM deoxycoformycin, 1 μM dipyridamole (these products prevent the reuptake and elimination of adenosine), 1 μM adenosine. The following were added to the other 4 samples in the following order: deoxycoformycin, dipyridamole and an Al receptor antagonist, 100 nM of DPCPX (1.3 dipropyl-8-cyclopentylxanthine), or an A2 receptor antagonist: 50 nM of CSC [8-(3-chlorostyryl) caffeine] and finally 1 μM adenosine.The last two samples were used as controls for the spontaneous incorporation of serotonin. All samples were incubated with 0.125 μCi (4.6 Bq) of [^{14}C]serotonin (5-hydroxy [^{14}C]tryptamine creatine sulfate, 55mCi/mmol, Amersham) 30 minutes at 37°C. After 10 min of centrifugation at 2,500g, aliquots (100 μl) of the supernatant containing non-incorporated [^{14}C]serotonin were counted for radioactivity.

[^{14}C] Serotonin Release From Platelets

Platelets were washed twice with phosphate buffered, saline (PBS, pH 7.4) containing 0.17 mM EDTA and 0.2% bovine serum albumine (BSA) and then resuspended in 1ml Tris buffer,

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pH 7.4, containing 20% foetal calf serum. The different reagents were added as for incorporation. 5 mM $CaCl_2$ and (Arg)8 vasopressin were then added to induce platelet aggregation.[14] After 5 min at 37°C, and a 10 min centrifugation at 2500 g, aliquots (100 µl) of supernatant containing released [^{14}C]serotonin were counted. Finally, we studied the effect of the adenosine concentration on the incorporation of serotonin by platelets in patients and controls, in the same conditions as for spontaneous serotonin incorporation.

Statistical Analysis

Analysis of variance (ANOVA)was used for adenosine plasma levels and [^{14}C] serotonin uptake comparisons between controls and patients. The Mann Whitney test was used to compare ADO plasma levels.

Results

One hour after the beginning of the migraine headache, there was a significant increase (mean 68%; Mann Whitney test; $p < .05$) in adenosine plasma levels in all patients. However, circulating adenosine levels measured in patients in migraine crisis-free periods were not significantly different ($p > .05$) from those noted in the controls (see Table 1).

When the adenosine concentration was less than or equal to 0.5 µM, serotonin incorporation by platelets was significantly lower in patients than in controls (ANOVA; $p = 0.049$, Figure and Table 2). At 2 µM, adenosine inhibited serotonin uptake by platelets in both migraine patients ($p = 0.042$) and in controls ($p = 0.012$, Figure). On the other hand, in the presence of both adenosine and CSC (an A2 receptor blocker), serotonin uptake by platelets (Table 2) was no different from spontaneous levels in both patients ($p = 0.06$) and in controls ($p = 0.07$). Finally, no significant change in vasopressin-induced serotonin release was observed in our experiments regardless of the product added (Table 2).

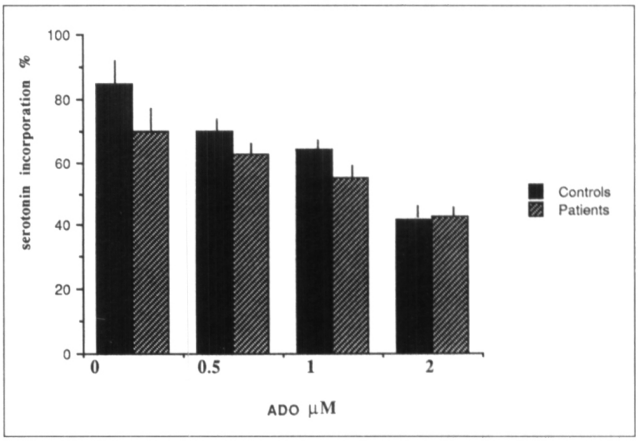

Figure: *Mean percentage and standard deviations of platelet incorporation of [^{14}C]-5HT as a function of adenosine concentration. (0, 0.5, 1 and 2 µM). [^{14}C]-5HT incorporation was significantly lower in migraine sufferers than in controls when the adenosine concentration in PRP was inferior or equal to 500 nM (Analysis of variance $p < 0.05$ in both cases).*

Discussion

The results of the present study lead to the following 3 conclusions:

1) There is an increase in circulating adenosine levels during migraine headache. This increase (mean 68%) probably has strong metabolic effects because it occurs near the low affinity adenosine receptors constant value (A2b).[4] This release of adenosine could participate in the vasodilation of the carotid territories since adenosine is a powerful vasodilator, even under physiological conditions.[6] This release could be secondary to an episode of oligemia resulting from the brain hypoperfusion which occurs during the first hour of the attack,[15] since adenosine is released by cells in cases of even mild oligemia and/or tissue hypoxia.[2] However, the presence

Table 2 : [^{14}C] Serotonin Incorporation and Release into Platelets.

	PATIENTS							
	Incorporation				Release			
Platelets number/ml (± SD)	Spontaneous	ADO	ADO + DPCPX	ADO + CSC	Spontaneous	ADO	ADO + DPCPX	ADO + CSC
290.000 (55.000)	69.5 (8.5)	54.9 (6)	35 (4.5)	73.9 (7.2)	49.4 (6)	44.2 (7)	52.4 (6)	48 (6.7)
	CONTROLS							
	Incorporation				Release			
Platelets number/ml (± SD)	Spontaneous	ADO	ADO + DPCPX	ADO + CSC	Spontaneous	ADO	ADO + DPCPX	ADO + CSC
310.000 (67.000)	84 (8.2)	63.9 (8)	39 (4.7)	68.3 (6.7)	42.2 (6)	55.5 (5.7)	45.8 (6)	51.7 (7)

Mean percentages of incorporation and release of platelet [^{14}C]-5HT in 12 patients suffering from common migraine and in 10 controls. Incorporation and release were done in platelet-enriched plasma samples, spontaneously in the presence of adenosine (1µm), adenosine and A1 receptor antagonists (100 nM DPCPX) or of adenosine and A2 receptor antagonists (50 nMCSC).

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of oligemia, in migraine without aura, is controversial. Another explanation of this release may be given by the hypothesis of Burnstock[16] who proposed that adenosine increase might be secondary to an increase in ATP which would then break down into adenosine. It is not clear which occurs first, the adenosine release or the headache, but it is assumed that the increase in circulating adenosine may keep up the cephalalgia, since intravenous adenosine can precipitate migraine crises.[8] The participation of adenosine release in headache could also explain the therapeutic effects of known adenosine antagonists such as caffeine. Adenosine release is not induced by pain, because a previous report has shown that plasma adenosine levels, is not modified in chronic pain except for neuropathic pain.[24] Stress does not increase adenosine plasma levels since, for example, the stress induced by lumbar puncture is not accompanied by such an increase.[24] Moreover, this increase could be specific to vascular headache since there is no modification in adenosine plasma level during facial neuralgia (unpublished data). However, to determine the specificity of our results, it will be interesting to evaluate blood adenosine levels in cluster headache, for example.

2) Adenosine at micromolar doses *in vitro* inhibits the uptake of platelet serotonin in both migraine sufferers and control subjects. It can be seen that, if there is an inhibition of serotonin uptake by platelets, this would increase the plasma levels of 5-HT in migraine and perhaps explain why there is increased elimination of 5-HT into the urine in migraine sufferers.[17]
3) This inhibition of platelet serotonin incorporation by adenosine is secondary to the activation of A2 receptors, since in the presence of adenosine and A2 blockers, serotonin incorporation becomes normal. Since adenosine receptors are primarily A2,[5] it is not surprising that no effect is observed in the presence of an A1 antagonist.

A number of neurotransmitters, neuropeptides and amines has been studied in migraine patients (for an overview see 18, 19). The majority of the research has involved circulating serotonin levels which increased, then decreased by elimination in urine, and may explain the vasomotor disturbances during crises.[18] Anomalies in nucleotide metabolism have also been reported: in the course of a migraine headache there is an increase in cAMP levels in the CSF[20] and the plasma.[21] In addition, it has been proposed that the increases in plasma levels of ATP and ADP observed during a migraine crisis are involved in the vasodilator effect and in pain, since these nucleotides can stimulate nerve endings.[16] Burnstock[22] suggested that following local vasospasm, the reactive hyperemia that occurs is due largely to ATP release from vascular endothelial cells, ATP acts on P2Y-purinoreceptors on endothelial cells to release nitric oxide (NO), which leads to vasodilatation. Among nucleosides, an elevation in plasma adenosine level in a small group of patients was reported[12] and the involvement of nucleosides in the pathophysiology of migraine has been previously suggested.[16] Adenosine has been also implicated in the genesis of other painful phenomena such as neuropathic pain[23,24] but to our knowledge, there are no data on the role of adenosine and its interaction with serotonin in migraine patients.

If these results are transposable *in vivo*, the use of A2 adenosine antagonists could be tried as a complementary treatment for migraine crises.

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